

Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment [☆]

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Abstract

Barley hull, a lignocellulosic biomass, was pretreated using aqueous ammonia, to be converted into ethanol. Barley hull was soaked in 15 and 30 wt.% aqueous ammonia at 30, 60, and 75 °C for between 12 h and 11 weeks. This pretreatment method has been known as “soaking in aqueous ammonia” (SAA). Among the tested conditions, the best pretreatment conditions observed were 75 °C, 48 h, 15 wt.% aqueous ammonia and 1:12 of solid:liquid ratio resulting in saccharification yields of 83% for glucan and 63% for xylan with 15 FPU/g-glucan enzyme loading. Pretreatment using 15 wt.% ammonia for 24–72 h at 75 °C removed 50–66% of the original lignin from the solids while it retained 65–76% of the xylan without any glucan loss.

Addition of xylanase along with cellulase resulted in synergetic effect on ethanol production in SSCF (simultaneous saccharification and co-fermentation) using SAA-treated barley hull and recombinant *E. coli* (KO11). With 3% w/v glucan loading and 4 mL of xylanase enzyme loadings, the SSCF of the SAA treated barley hull resulted 24.1 g/L ethanol concentration at 15 FPU cellulase/g-glucan loading, which corresponds to 89.4% of the maximum theoretical yield based on glucan and xylan.

SEM results indicated that SAA treatment increased surface area and the pore size. It is postulated that these physical changes enhance the enzymatic digestibility in the SAA treated barley hull.

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1. Introduction

There has been growing interest in using barley grain as a feedstock for fuel ethanol. Barley is currently being used for ethanol production in Europe but not in the US. Barley has some advantages as a corn substitute for ethanol production outside the Corn Belt, particularly on the East

Coast, the upper Midwest, and the Northwest (USDA-NASS, 2006). North America grows approximately 14% of the world annual production of barley (Kim and Dale, 2004). Most fuel ethanol in the US is corn-based. Hence most production facilities are located in the Corn Belt, not on either of the coasts where demand for ethanol is high (Hsu, 1996). Barley hull, obtained as a low-value by-product of barley (starch) ethanol facilities, represents a pre-collected source of lignocellulosic biomass that could be utilized on site for “cellulosic” ethanol production. Transportation of biomass to an ethanol production facility is one of the main costs in most cellulosic ethanol production systems (Mahmudi, 2005; Searcy et al., 2007). Using a pre-collected form of biomass, as in barley hull, could result in transportation and energy cost savings.

[☆] Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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Another difficulty in utilization of biomass is to maintain year-round supply of feedstock. Agronomically, winter barley fits extremely well into a three-crop two-year rotation with corn and soybeans. Barley is harvested earlier than wheat, allowing a farmer to plant (double crop) soybeans earlier, which can result in good maturity and high yields of the soybeans harvested later that same year. Barley grows well in many areas where corn does not; therefore, it may become a financially cost-effective ethanol feedstock for these regions (USDA-NASS, 2006). Furthermore, the composition of barley hull indicates that about 70% of the weight is carbohydrate which could potentially be utilized for ethanol production.

For fuel ethanol production, pretreatment has been studied as a key step for the effective utilization of lignocellulosic biomass feedstock, due to its recalcitrant nature. Part of the effect of pretreatments is the removal of lignin, a constituent that is known to inhibit saccharification enzymes and fermentative microorganisms (Chang and Holtzapfel, 2000; Mooney et al., 1998; Schwald et al., 1988; Cowling and Kirk, 1976). The barley hull is also quite abrasive on processing equipment and makes up a considerable amount of a hulled barley kernel, up to 10–15% of the grain weight. A pretreatment that can reduce the rigidity of this material is therefore desired.

Ammonia as a pretreatment reagent has many advantages for an effective delignification as well as swelling of biomass. Pretreatment methods using aqueous ammonia have been studied for the purpose of ethanol production (Kim and Lee, 2007, 2006, 2005a, 2005b; Kim et al., 2003). Among them, the soaking in aqueous ammonia (SAA) at low temperature retains the hemicellulose in the solids by minimizing the interaction with hemicellulose during treatment, which was reported as a feasible approach to increase the fermentation yield and simplify the bioconversion scheme (Kim and Lee, 2007, 2005b). Retained xylan can usually be hydrolyzed to fermentable pentoses by most commercial cellulase and xylanase mixtures (Kim and Lee, 2007, 2005b). In the study, barley hull was soaked in 15–30 wt.% aqueous ammonia for extended periods of time at 30–75 °C. This study was focused on evaluation of the SAA method as a pretreatment method of barley hull for the production of ethanol and the effect of additional xylanase on SSCF reaction of SAA-treated barley hull. Enzymatic saccharification was conducted to evaluate the pretreated hull's potential for bioconversion to fuel ethanol and/or for use as a ruminant (dairy and beef cattle) feed component with enhanced digestibility.

2. Methods

2.1. Materials

Nomini, a six-row winter hulled barley grown and harvested in Virginia in 2004, was used in this study. The barley was dehulled by a roller mill and aspirator resulting in a

purified hull fraction which contained only about 12.1 wt.% of residual starch. Separated barley hull was put through a two-step enzymatic destarching process to remove the residual starch in order to avoid interference with glucose from cellulose during experiments. It is probable that in a future commercial process, separated hull would be subjected to the destarching process, and then the recovered residual starch would eventually be combined with the main starch liquefaction stream. Enzymes and the conditions for the treatment used were as follows:

- Amylase (Spezyme Fred, Genencor International Inc. Lot #107-02285-003), pH 6.0, 80 °C, 60 min.
- Glucoamylase (Optidex L-300, Genencor International Inc. Lot #105-01232-001), pH 4.5, 55 °C, 60 min.

Cellulase enzyme, GC-220 (Genencor International Inc., Lot #301-04232-162), was obtained from Genencor International. The average activity of the enzyme was 45 filter paper unit (FPU)/mL and the protein content was 184 mg/mL. Activity of β -glucosidase (Novozyme 188 from Novo Inc., Lot No. 11K1088) was 750 CBU/mL. Xylanase enzyme, Multifect-Xylanase (Genencor International Inc) was used for the SSCF tests and the protein content was 40 mg/mL.

Avicel® PH-101, microcrystalline cellulose (MCC), was purchased from Sigma–Aldrich (Sigma Cat. No. 11365, Lot No. 1094627-54804207). Avicel was used as a reference in the enzymatic digestibility test for cellulose, because it is nearly pure cellulose (~97%) and has no lignin with uniform quality in any batch.

Recombinant *Escherichia coli* ATCC® 55124 (KO11) was employed for the SSCF tests. LB medium (Sigma Cat. No. L-3152) was used for the growth of KO11, which contained 1% tryptone, 0.5% yeast extract, 1% NaCl, and 40 mg/L chloroamphenicol.

2.2. Experimental setup and operation

For the pretreatment using SAA, destarched barley hull was treated with 15 or 30 wt.% of aqueous ammonia in screw-capped laboratory bottles (pyrex bottles) at 30–75 °C for 12 h to 77 days with no agitation. Solid-to-liquid ratio of 1:12 was applied. After soaking, the solids were separated by filtering, washed with deionized (DI) water until its pH was around 7.0, and then subjected to the solid compositional analyses and enzymatic digestibility tests. Acid insoluble lignin, carbohydrate content, and digestibility were all determined following NREL Chemical Analysis and Testing Standard Procedure (NREL, 2004).

2.3. Digestibility tests

The enzymatic digestibility of barley hull was determined in duplicate according to the NREL Chemical Analysis and Testing Standard Procedure (NREL, 2004). The conditions of the enzymatic digestibility tests were 50 °C

and pH 4.8 (0.05 M sodium citrate buffer) on a shaker bath agitated at 150 rpm. Enzyme loadings of 15 (61 mg of protein) and 30 FPU (122 mg of protein) of GC-220/g-glucan supplemented with 30 CBU of β -glucosidase (Novozyme 188)/g-glucan were used. The initial glucan concentration was 1% (w/v) based on 100 mL of total liquid and solid. All the samples used in the digestibility tests were wet samples as collected from various pretreatments. The 250 mL screw-capped Erlenmeyer flasks containing the enzyme hydrolysis preparations were placed in an incubator shaker (Lab-line, 4827F, Dubuque, IA). Samples were taken periodically (6, 12, 24, 48, 72 and 96 h) and analyzed for glucose, xylose, and cellobiose content using HPLC (NREL, 2004). Total released glucose (or xylose) after 72 h of hydrolysis was used to calculate the enzymatic digestibility. Avicel® PH-101 and untreated barley hull were put through the same procedure as a reference and control, respectively.

2.4. Simultaneous saccharification and co-fermentation (SSCF)

A 250 mL Erlenmeyer flask was used as the bioreactor. It was shaken in the incubator shaker (New Brunswick Scientific, Innova-4080) at 38 °C with 150 rpm. Into a 100 mL working volume of liquid, treated barley hull sample was introduced to reach 3% w/v glucan content in the reactor. Avicel® PH-101 was put through the same procedure as the control. The SSCF runs were performed with buffer without external pH control, starting at pH 7.0 at the beginning of the fermentation and gradually decreasing to pH 5.5 at the end. The loading of cellulase enzyme (GC-220) was 15 or 30 FPU/g-glucan, and that of β -glucosidase (Novozyme 188) was 30 CBU/g-glucan.

The ethanol yield in SSCF test was calculated as follows:

$$\text{Theoretical maximum ethanol yield(\%)} = \frac{\text{Ethanol produced(g) in reactor}}{\text{Initial Sugar(g) in reactor} \times 0.511} \times 100$$

Note: Sugar is interpreted as glucose plus xylose in the SSCF work.

2.5. Analytical methods

The solid samples, such as treated/untreated barley hull, Avicel® PH-101, etc., were analyzed for sugar and Klason lignin following NREL Chemical Analysis and Testing Standard Procedures (NREL, 2004). Each sample was run in duplicate. Sugars were determined by HPLC using a Bio-Rad Aminex HPX-87P column and a refractive index detector (NREL, 2004).

For the SSCF tests, HPX-87P and 87H columns were used to measure the sugar content and ethanol, respectively. An YSI 2300 Glucose/Lactate analyzer (YSI Incorporated, Yellow Springs, OH) was used for rapid analysis

of glucose during inoculums preparation. A refractive index detector was used for HPLC analysis.

2.6. SEM

A Quanta 200 FEG Environmental Scanning Electron Microscope (FEI Co. Inc., Hillsboro, Oregon) was used to take images of treated and untreated samples. The samples were dried and mounted on aluminum sample stubs. Then, the mounted samples were coated with a thin layer of gold.

3. Results and discussion

Table 1 summarizes the initial compositions of destarched barley hull and microcrystalline cellulose, Avicel. This showed that approximately 70 wt.% of barley hull is potentially available carbohydrate, which can be converted to ethanol by pentose and hexose fermenting organisms. It was noticed that the amount of the second most abundant polysaccharide, xylan (30.5%) is almost equivalent to the amount of glucan in the de-starched hull material (33.6%). Also 6.0% of arabinan is an additional amount of potentially available carbohydrate resource. The strategy in this study is to retain the hemicellulose (pentoses, xylose and arabinose) in the solid during pretreatment, which then can be utilized using co-fermentation of hexoses and pentoses derived from the solid portions only. This strategy also eliminates the generation of soluble sugar fractions which is the case for most other pretreatments using high temperature conditions generating various toxic components, such as furfural, HMF (hydroxymethyl furfural) and other decomposition products (Fein et al., 1984; Hahn-Hägerdal et al., 1994; Björling and Lindman, 1989; Sanchez and Bautista, 1988; Tran and Chambers, 1986; Watson et al., 1984). Since the detoxification and cleaning of these hydrolysates is a high cost step, exclusion of these fractions can result in a simple conversion scheme and operational cost savings.

Table 1
Initial compositional analysis of destarched barley hull and Avicel^a

Components	Barley hull (destarched) (%)	Avicel ^b (%)
Glucan	33.6	97.0
Xylan	30.5	2.2
Galactan	0.6	0.2
Arabinan	6.1	1.5
Mannan	Trace	Trace
Lignin ^c	19.3	–
Ash	3.6	–
Starch	<0.8	–
Total	93.6	100.8

^a Data in the table are based on oven dry samples.

^b Avicel® PH-101 Micro crystalline cellulose, Sigma Cat. No. 11365, Lot No. 1094627-54804207.

^c Acid insoluble lignin.

Table 2
Effect of reaction time and temperature on the composition and enzymatic digestibility in SAA-treated barley hull at low temperature^a

Treatment time and temperature (weeks)	SR ^b (%)	Lignin ^c (%)	Delignification (%)	Solid composition ^d		Enzymatic digestibility ^e	
				Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)
Untreated	–	19.3	–	33.6	30.5	7.3	5.5
30 °C ^a							
1 week	89.2	13.4	30.6	33.6	30.0	54.5	38.6
2 weeks	85.2	12.1	37.3	33.5	29.0	62.0	45.3
7 weeks	78.0	10.8	43.5	33.3	25.7	68.9	54.6
11 weeks	76.3	9.5	50.5	33.5	25.2	68.3	53.9
60 °C ^a							
12 h	85.1	13.2	31.6	33.1	29.8	57.6	41.8
24 h	79.2	11.2	42.0	32.6	26.0	66.4	47.4
75 °C ^a							
24 h	72.0	9.6	50.3	32.8	23.4	75.7	56.0
48 h	65.0	7.5	61.1	33.1	20.5	82.7	62.5
72 h	63.4	6.9	64.3	33.0	20.0	83.0	62.8
144 h	62.6	6.6	65.8	33.7	19.7	85.0	64.0

^a Data (lignin, glucan, and xylan contents) in the table are based on the oven dry untreated biomass. Pretreatment conditions: 30 wt.% at 30 °C, 15 wt.% at 60 °C and 15 wt.% at 75 °C, and a 1:12 solid:liquid ratio (based on wt.).

^b SR stands for solid remaining after reaction.

^c Acid insoluble lignin.

^d For instance, when the data says that the solid contains 33.6% glucan based on the oven dry untreated biomass, which means the remaining solids (89.2% of untreated weight) contains 37.7% glucan.

^e Digestibility at 72 h, enzymatic hydrolysis conditions: 15 FPU/g-glucan, pH 4.8, 0.05 M citrate buffer, digestibility at 50 °C and 150 rpm.

3.1. Composition change and enzymatic digestibility at various reaction times and temperatures

Table 2 summarizes the composition changes in solids upon various reaction conditions.

At first attempt at low temperature, the soaking in aqueous ammonia pretreatment was tested at 30 °C during four different reaction times covering 1–11 weeks (Table 2). In this study, barley hull was soaked in 30 wt.% of aqueous ammonia at 30 °C keeping a 1:12 solid:liquid ratio. The low treatment temperature (at 30 °C) was chosen since the lower heat input may reduce the interaction between ammonia and hemicellulose. Lignin removal was 31–51% and xylan was solubilized in the range of 217% over the extended period. Approximately 31% of lignin was removed in 1 week. The glucan and xylan content was well preserved during the treatment period (Table 2).

Fig. 1 shows the enzymatic digestibilities of pretreated barley hull with 15 and 30 FPU/g-glucan enzyme loading. The digestibility number on the y-axis represents the percent saccharification (percent of the theoretical glucose yield). After SAA pretreatment, the 72-h enzymatic digestibility of glucan was enhanced from 8% to a maximum of 78% with 30 FPU/g-glucan (Fig. 1) and from 7% to 68% with 15 FPU/g-glucan enzyme loading (Table 2). With 15 FPU/g-glucan enzyme loading (Table 2), 39–55% of the xylan in the treated barley hull was also hydrolyzed by cellulase enzyme (GC-220) within 72 h hydrolysis time. However, these results may find little interest in industry due to the long reaction times and low digestibilities. Even though initial hydrolysis rates of treated barley hulls (7 and 11 weeks SAA-treated)

are much faster than that of Avicel, which has no chemical barriers such as lignin and hemicellulose, the final yields, however, are much lower than Avicel (Fig. 1).

The temperature of treatment was increased in order to reduce the treatment time. The pretreatment at 60 °C for 6–24 h with 15 wt.% ammonia was initially attempted (Table 2). The data indicated that 60 °C reaction temperature for this time interval results in low digestibilities. The final enzymatic digestibilities (at 72 h) at 6 and 24 h are only 57.6% and 66.4% with 15 FPU/g-glucan enzyme loading, respectively.

At a higher temperature of 75 °C, four different reaction times (24, 48, 72 and 144 h) were applied, keeping a 1:12 solid:liquid ratio and 15 wt.% ammonia concentration. At this temperature level, a significant compositional change occurred in lignin and xylan. Approximately half of the lignin was removed within 24 h. Delignification reached 61% after 48 h and 66% after 144 h. Table 2 also shows that most of the compositional changes occur within 48 h. Xylan dissolution was 23% after 24 h, 33% after 48 h, and 35% after 144 h. The glucan content was well preserved showing no significant changes over the entire treatment time. Therefore, increasing the soaking time beyond 48 h had no significant effect on the solid compositions and final enzymatic digestibilities (Table 2).

The enzymatic digestibilities of glucan in the SAA-treated barley hull at 75 °C are shown in Fig. 2. With 15 FPU/g-glucan enzyme loading, the 24 h SAA-treated barley hull showed 72 h-digestibility of 76% for glucan, whereas the 48, 72 and 144 h samples showed 83%, 83% and 85% with 15 FPU/g-glucan, respectively (Fig. 2a).

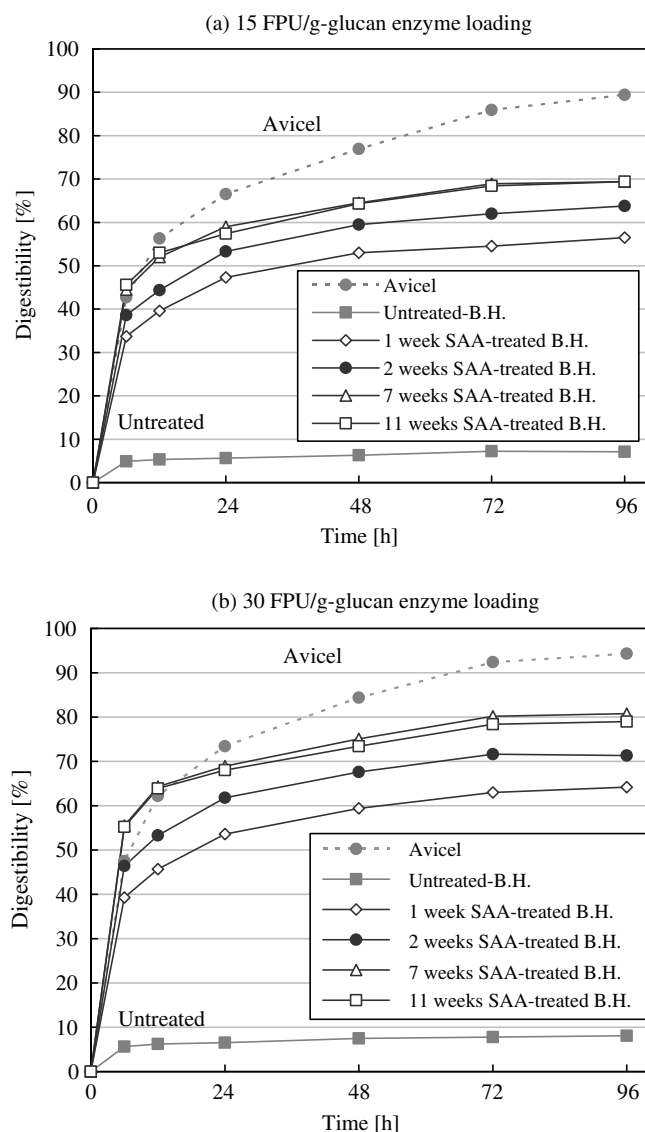


Fig. 1. Glucan digestibility of SAA treated (at 30 °C) and ground barley hulls. *Note:* 15 or 30 FPU of cellulase/g-glucan, 30 CBU of β -glucosidase/15 FPU-cellulase, 50 °C, 150 rpm; Pretreatment conditions: 15 wt.% ammonia concentration, 30 °C of reaction temperature, 1–11 weeks of reaction time, 1:12 of solid:liquid ratio (based on weight).

Increasing the enzyme loading from 15 FPU/g-glucan to 30 FPU/g-glucan slightly increased the glucan digestibility (81–87% at 72 h). The 24 h SAA-treated barley hull showed 72 h-digestibility of 81%, whereas the 48, 72 and 144 h sample showed 86%, 87% and 87% with 30 FPU/g-glucan, respectively (Fig. 2b).

Increasing pretreatment time beyond 48 h did not enhance the digestibility significantly (Fig. 2). Moreover, enzymatic digestibilities with 30 FPU were not much higher than those with 15 FPU/g-glucan enzyme loading. Therefore, 48 h and 15 FPU/g-glucan were chosen as the optima for glucan digestibility considering energy input, equipment cost, and enzyme dosage.

The enzymatic digestibility results for xylan in SAA-treated barley hull are shown in Fig. 3. The xylan digestibil-

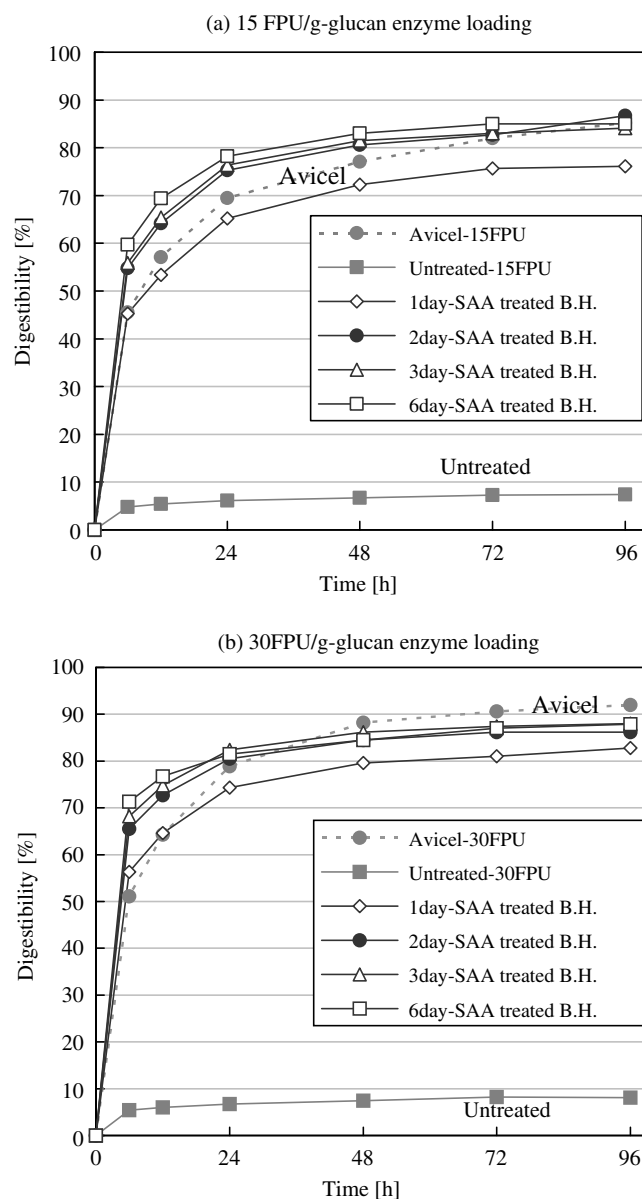


Fig. 2. Glucan digestibility of SAA treated (at 75 °C) barley hull. *Note:* 15 or 30 FPU of cellulase/g-glucan, 30 CBU of β -glucosidase/15 FPU-cellulase, 50 °C, 150 rpm. Pretreatment conditions: 15 wt.% of ammonia concentration, 75 °C of reaction temperature, 24–144 h of reaction time, and 1:12 of solid:liquid ratio (based on wt.).

ities at 72 h were in the range of 56–64% with 15 FPU/g-glucan and 62–68% with 30 FPU/g-glucan (Fig. 3a and b). With 15 FPU/g-glucan, the observed xylan digestibilities of 56–64% are much lower than the corresponding 76–85% for glucan digestibilities. SAA treatment for 48 h resulted in 83% for glucan and 63% for xylan with 15 FPU of cellulase/g-glucan enzyme loading. The xylanase activity in cellulase GC-220 in this study, while it is quite substantial, does not match the cellulase activity on glucan. Additional xylan components in SAA treated barley hull could potentially be released in the monomeric form with additional xylanase enzymes.

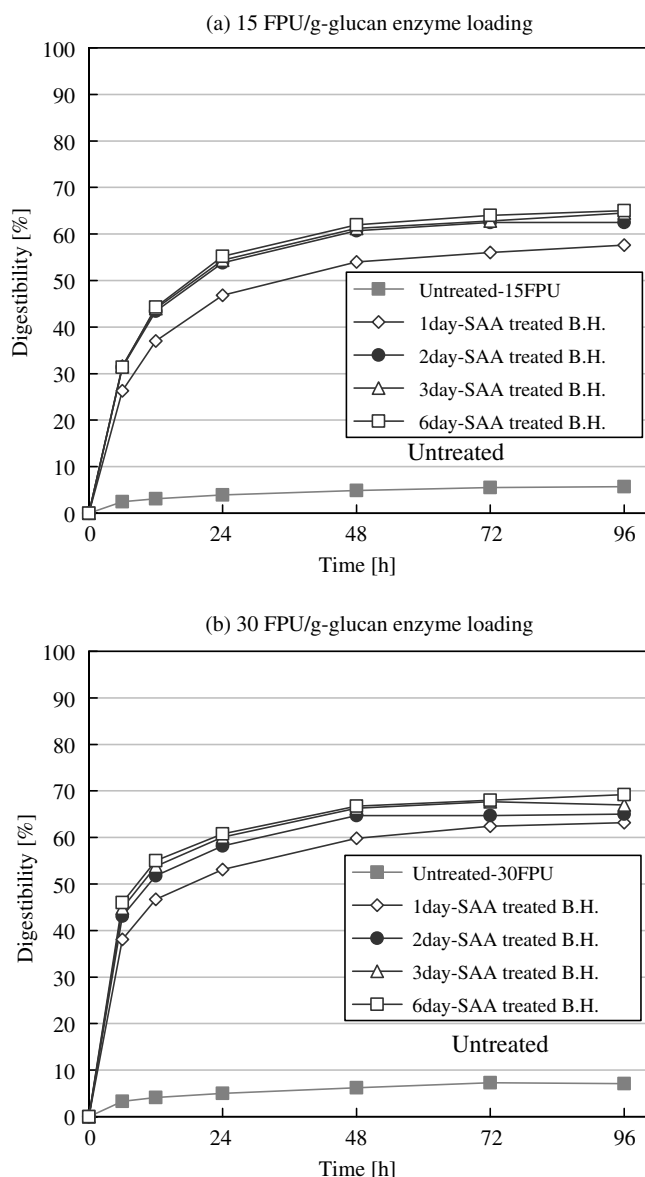


Fig. 3. Xylan digestibility of SAA treated barley hull upon pretreatment time (at 75 °C). Note: 15 or 30 FPU of cellulase/g-glucan, 30 CBU of β -glucosidase/15 FPU-cellulase, 50 °C, 150 rpm. Pretreatment conditions: 15 wt.% of ammonia concentration, 75 °C of reaction temperature, 24–144 h of reaction time, and 1:12 of solid:liquid ratio (based on wt.).

The best treatment conditions observed in this study were determined to be 15 wt.% ammonia, 75 °C reaction temperature, and 48 h treatment time. Under these treatment conditions, the destarched barley hull retained 67% of the xylan and removed 61% of the lignin. The enzymatic digestibility was enhanced from 7% to 83% for glucan with 15 FPU/g-glucan enzyme loading, and 63% of the xylan in the treated barley hull was also hydrolyzed by cellulase enzyme (GC-220). Palmarola-Adrados et al. (2005) previously reported that pretreatment of barley husks using 0.5% H_2SO_4 at 200–205 °C improved the enzyme saccharification yields significantly. The results using SAA are comparable to those of 88% for glucan and 55% for xylan

reported in their previous study even though the pretreatment and test methods used were different in each study. Compared to the dilute acid pretreatment methods reported, the present method uses much simpler equipment, requires less energy, may produce less fermentation inhibitors such as furfural, and results in most of the hemicellulose and cellulose fractions being retained in the solid fraction for ease of handling.

3.2. SSCF of SAA-treated barley hull

The SAA-treated barley hull was subject to the simultaneous saccharification and co-fermentation (SSCF) using recombinant *E. coli* ATCC® 55124 (KO11) and GC-220 cellulase enzyme. The SAA conditions for the barley hull were: 15 wt.% ammonia, 70 °C, 72 h of treatment time, and 1:12 of solid-to-liquid ratio. At 3 w/v% of glucan loading case, the theoretical maximum ethanol concentration produced can be calculates as follows:

SAA-treated barley hull in the 100 mL working volume reactor has 3 g of glucan + 1.6 grams of xylan, thus it can reach 27.0 g/L of ethanol concentration.

Fig. 4 presents ethanol concentrations in the SSCF, and the maximum ethanol concentration reached 19.6 g/L for 30 FPU and 18.9 g/L for 15 FPU enzyme loading after 72 h. In other words, ethanol yields were 73.6% and 71.6% of the maximum theoretical yield based on glucan and xylan in the pretreated barley hull.

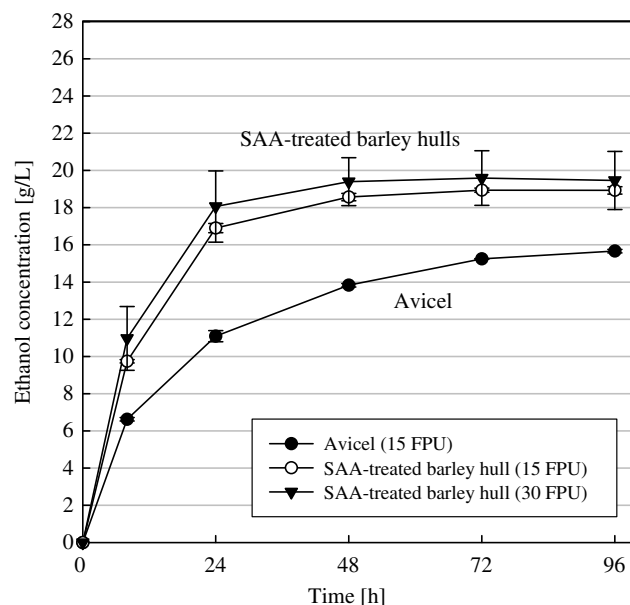


Fig. 4. Simultaneous saccharification and co-fermentation (SSCF) of SAA-treated barley hull. Note: Microorganism: *Escherichia coli* ATCC® 55124; substrate: 3% w/v glucan loading/100 mL working volume; SAA-treated barley hull (15 wt.% of ammonia conc., 70 °C of reaction temp., 72 h of reaction time, and 1:12 of solid:liquid ratio); SSCF: 15 or 30 FPU of GC-220/g-glucan; 30 CBU of Novozyme 188/g-glucan; LB medium; anaerobic condition; 38 °C, 150 rpm.

3.3. Additional xylanase effects in SSCF of SAA-treated barley hull

SSCF test using cellulase alone in Fig. 4, the ethanol yields were 114.2% and 111.1% based on input glucan only, and it confirmed that the KO11 strain converted the xylose component as well as glucan component to ethanol. It also indicated that the some of glucan and xylan were not hydrolyzed in the reactor, but the each saccharification yield from glucan and xylan was unknown. In Fig. 3, enzymatic digestibility test for xylan component has shown

about 63% of digestibility, which was substantially lower than glucan digestibility (83%). The main purpose of the SSCF is to convert both hexose and pentose in a single reactor.

Most of commercial cellulase enzyme has its xylanase activity as well, but it was found that its xylanase activity was not sufficient to hydrolyze the total xylan component in the SAA-treated barley hull (Figs. 3 and 4). In order to investigate effect of additional xylanase, Multifect xylanase (Genencor International Inc., Palo Alto, CA) was added to the SSCF reactor. Fig. 5 presents the ethanol concentration profile of SAA-treated barley hull with addition of xylanase. Three different xylanase loadings were tested at 1, 2, and 4 mL of xylanase/reactor.

Addition of xylanase along with cellulase brought about synergetic hydrolysis of SAA-treated barley hull, thus it increased the ethanol yield in SSCF (Simultaneous Saccharification and Co-Fermentation). With 3% w/v glucan loading and 4 mL of xylanase enzyme loadings, the SSCF of the SAA treated barley hull resulted 24.1 g/L and 23.8 g/L ethanol concentration at 15 and 30 FPU cellulase/g-glucan loading, respectively, which corresponds to 89.4% and 88.4% of the maximum theoretical yield based on glucan and xylan. As seen in Fig. 5a and b, the higher ethanol concentration was obtained with higher xylanase enzyme loading. In both Fig. 5a and b, the xylanase additions which were more than 2 mL of xylanase/reactor resulted in nearly the same about similar final ethanol concentrations. It was speculated that the synergetic hydrolysis effect on the biomass could lower the cellulase enzyme loadings, expecting the same ethanol yields.

3.4. SEM

Physical changes for various cellulosic materials were studied using SEM (Fig. 6). The pictures in the left hand columns (Fig. 6a–d) are of the materials before pre-treatment and the pictures on the right hand column (Fig. 6a-1–d-1) are of the residual solids after enzyme hydrolysis.

Fig. 6c shows that in SAA treated barley hulls, there are separated and modified fibers present on most of the biomass surfaces. Fig. 6b indicates that the surface of untreated barley hulls is rigid and has many phytoliths, which are decay-resistant silica bodies that protect the fibers against biodegradation. However, the fibers of SAA-treated samples are seen to have been separated and peeled off from the initial connected structure and are fully exposed. Thus, SAA treatment altered the biomass structure significantly. It is speculated that the improvements in enzyme hydrolysis in the SAA pretreated samples are due to the increased surface area and the presence of pores that can be accessed by enzymes. Additional study with residual solids after cellulase enzyme hydrolysis (after 96 h, Fig. 6a-1–d-1) shows that fiber skeletons remained after enzyme hydrolysis (Fig. 6a-1 and c-1) and were observed in Avicel and SAA-treated barley hull.

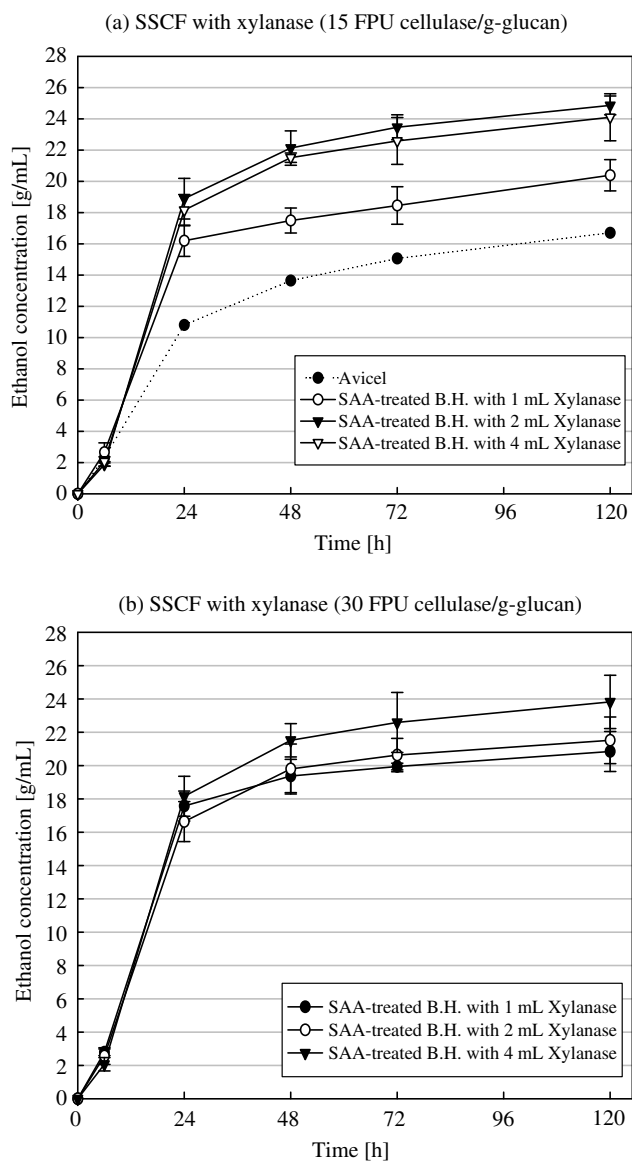
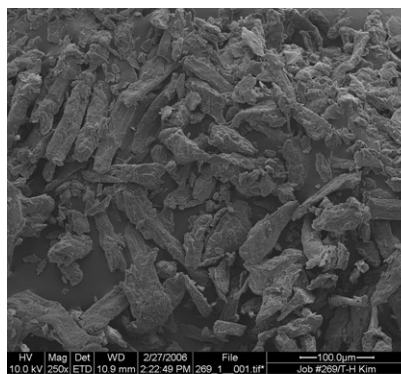
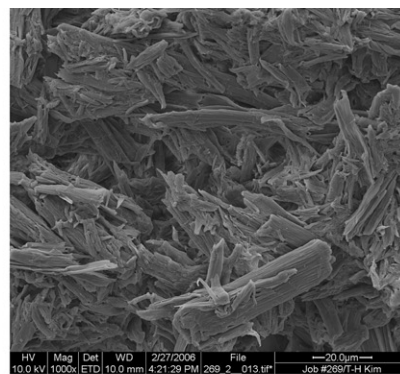


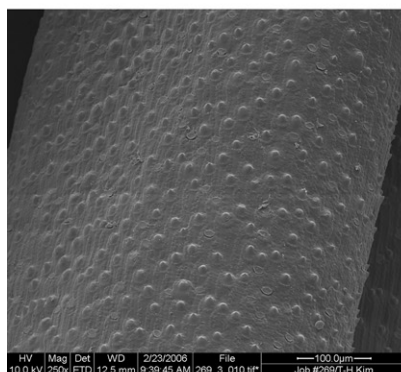
Fig. 5. Xylanase effect in simultaneous saccharification and co-fermentation (SSCF) of SAA-treated barley hull. Note: Microorganism: *Escherichia coli* ATCC® 55124; substrate: 3% w/v glucan loading/100 mL working volume; SAA-treated barley hull (15 wt.% of ammonia conc., 70 °C of reaction temp., 72 h of reaction time, and 1:12 of solid:liquid ratio); SSCF: 15 or 30 FPU of GC-220/g-glucan; 30 CBU of Novozyme 188/g-glucan; LB medium; anaerobic condition; 38 °C, 150 rpm.



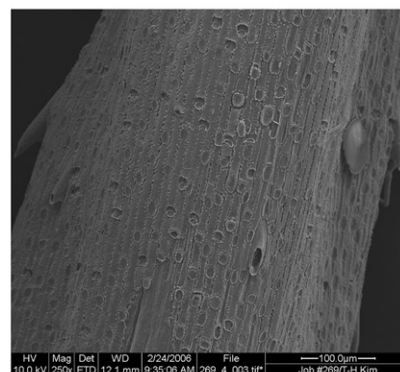
(a) Avicel



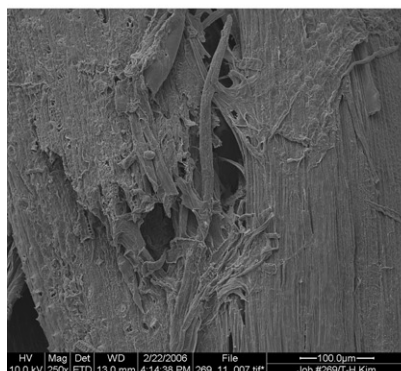
(a)-1 Avicel (residues after enzyme hydrolysis)



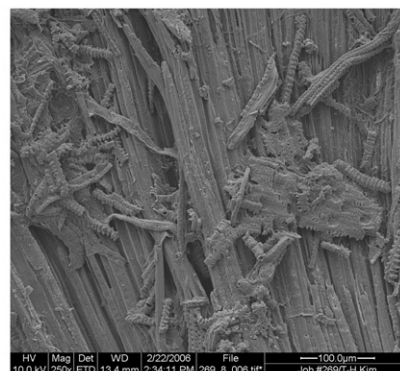
(b) Untreated barley hull



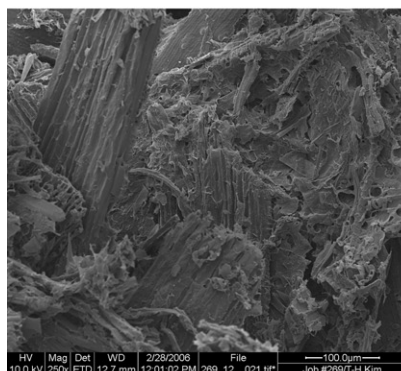
(b)-1. Untreated barley hull (residues after enzyme hydrolysis)



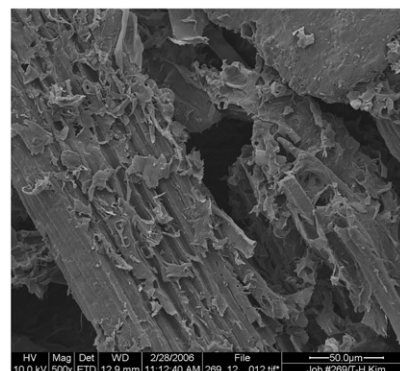
(c) SSA treated-barley hull



(c)-1. SSA treated-barley hull (residues after enzyme hydrolysis)



(d) Ground barley hull using Supraton



(d)-1) Ground barley hull using Supraton (residues after enzyme hydrolysis)

Fig. 6. SEM pictures of various cellulosic materials.

4. Conclusion

Based on current study, the best pretreatment conditions for barley hull can be defined as 15 wt.% aqueous ammonia, 75 °C of reaction temperature, 48 h reaction time, and a 1:12 (based on wt.) solid:liquid ratio. Using this best treatment, about 66% of lignin was solubilized, 67% xylan and all of the glucan in the washed solids were retained. Retention of glucan and xylan in the solids fraction is a feasible and simple strategy for biomass bioconversion. SAA pretreatment enhanced the enzymatic saccharification yields (digestibility) significantly, which were 83% for glucan and 63% for xylan with 15 FPU of cellulase/g-glucan enzyme loading. Higher lignin removal appears to be the primary reason for the increased enzymatic digestibility. It is speculated that aqueous ammonia treatment causes swelling as well as physical change due to a significant lignin removal.

With 4 mL of additional xylanase in SSCF, high ethanol yield (89.4% and 88.4% of the maximum theoretical yield based on glucan and xylan) was achieved at 15 and 30 FPU/g-glucan enzyme loadings, respectively.

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